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ENVIRONMENTAL CONTAMINANTS PROGRAM
ON-REFUGE INVESTIGATIONS SUB-ACTIVITY

***Mercury on national wildlife refuges as a threat to long-term viability of saltmarsh and Nelson's sparrows in the face of climate induced sea level rise**

by

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Abstract

Nelson's and saltmarsh sparrows (*Ammodramus nelsoni* and *A. caudacutus*) have recently been recognized as separate species, and because of their limited distributions and the susceptibility of their wetland habitats to climate change, these two new species are of conservation concern. Both species are known to bioaccumulate mercury at the New England, USA breeding sites where their ranges overlap, with the saltmarsh sparrow reported to have twice the concentration of blood total mercury. In this study we sampled both species on their shared wintering grounds, and documented that mercury exposure is lower than that reported for the breeding range, with saltmarsh sparrow blood mercury approximately three times higher than its congener. Feather mercury, which is incorporated on the breeding grounds, confirmed that saltmarsh sparrows had been exposed to twice as much mercury as Nelson's sparrows during the previous breeding season. A comparison of stable isotopes of nitrogen and carbon suggests that the higher exposure of saltmarsh sparrows is not due to feeding at a higher trophic level, as previously hypothesized, but rather may be related to a difference in the carbon source at the base of each species' food chain. This study, along with recently published data from both species on additional breeding and wintering grounds, provides a more complete picture of relative mercury exposure. Saltmarsh sparrows are exposed to mercury levels that warrant concern, with the highest exposure being during the breeding season. Areas set aside for the long-term conservation of this species should be carefully assessed for mercury bioaccumulation.

Keywords: Chesapeake Bay, mercury, Nelson's sparrow, saltmarsh sparrow, stable isotopes, wintering ground, Parker River National Wildlife Refuge (NWR), Rachel Carson NWR, Chincoteague NWR, Eastern Shore of Virginia NWR, Back Bay NWR, Eastern Virginia Rivers Complex

Table of Contents

List of Tables

List of Figures

List of Acronyms/Abbreviations

micrograms per liter (μl)
National Wildlife Refuge (NWR)
parts per million (ppm)
standard deviation (sd)

Introduction

Saltmarsh sparrows (*Ammodramus caudacutus*) are a species of special concern for the U.S. Fish and Wildlife Service (Service) and are on the Audubon Society's "watchlist," as well as numerous state lists of vulnerable species. They have been the focus of conservation concern because of their limited habitat and geographic range, occurring only in tidal marshes in the eastern United States with a breeding range limited to New England and the Mid-Atlantic. Because their region of occurrence and habitat have been heavily impacted by humans for two centuries, this species is dependent on publicly-owned wetlands for survival. Even on the many tidal marshes protected by the National Wildlife Refuge (NWR) system, the future holds abundant threats for this vulnerable species, primarily climate-induced sea-level rise (Erwin et al. 2006), but also invasion by non-native *Phragmites* reed and other forms of wetland degradation. The subject of this proposal is evaluation of a recently identified potential threat: contamination of the saltmarsh sparrow's limited habitat on NWRs by mercury from upwind and upstream.

Mercury is a toxic heavy metal that, when added to the environment by humans, biomagnifies in food chains as methylmercury and becomes a potential stressor for wildlife populations (Wolfe et al. 1998). Primarily thought to be a problem for fish-eating wildlife such as otter, loons and eagles, it has recently become clear that mercury can also be a problem for many other species, such as songbirds, that eat invertebrates in areas impacted by mercury (Cristol et al. 2008). Because methylation of inorganic mercury is enhanced by warmer temperatures, increased variability in water levels, and erosion, it is likely that mercury contamination will be exacerbated by global climate change in eastern saltmarshes. A study to be published soon by the USGS indicates that marine mercury has increased by 30% in recent decades and is likely to increase more in the future. Adequate management plans for long-term survival of this species on refuges requires rapidly addressing the interaction of mercury and climate change.

Several pilot studies have already established that saltmarsh sparrows are accumulating mercury. Shriver et al. (2006) found that saltmarsh sparrows accumulated more mercury than the very similar Nelson's sparrow, with which it shares some New England breeding sites, and suggested that its larger bill led saltmarsh sparrows to feed higher on the food web and accumulate more mercury as a result. Lane et al. (2008) compared mercury exposure and reproductive success of saltmarsh sparrows on two NWRs in New England (Parker River NWR, Massachusetts and Rachel Carson NWR, Maine) from 2004-2007 and found that birds at Parker River had higher mercury levels and a trend towards lower reproductive success. These two studies from the breeding grounds suggest that mercury accumulation in some individuals can reach levels approaching those known to affect populations of other bird species (Brasso and Cristol 2008, Evers et al. 2008), is specific to particular geographic areas (Parker River was far worse than Rachel Carson), and is worse in this species than even closely related species sharing the same habitat (Nelson's sparrow).

An additional pilot study was conducted on the Eastern Shore of Virginia NWR wintering range in 2008, confirming that even on the wintering grounds saltmarsh sparrows accumulate more blood mercury, by a factor of 5, than the similar Nelson's sparrows, (saltmarsh: 0.58 ± 0.15 parts per million (ppm), n = 10; Nelson's: 0.12± 0.04 ppm, n = 14; unpublished data, D. A. Cristol and B. D. Watts, College of William & Mary). More importantly, even with only 10 individuals sampled from the Eastern Shore of Virginia NWR, this pilot study detected some individuals with blood levels of mercury that could potentially cause adverse effects (saltmarsh sparrow range: 0.32-0.81 ppm), especially in conjunction with other stressors. To summarize, mercury from external sources is accumulating at potentially harmful rates in vulnerable populations of saltmarsh sparrows on breeding and wintering sites inside national wildlife refuges. It is important to determine whether mercury is a factor to be considered in long-term management plans for this species, which will be severely impacted by climate-induced sea level rise.

The objectives of the study were to:

Determine the proportion of individuals wintering at four NWRs (Chincoteague, Eastern Shore of Virginia, Back Bay and Eastern Virginia Rivers Complex) that are receiving mercury exposure at a level that puts them at risk for reduced fitness (~1 ppm in wet weight blood based on extrapolation from the incomplete literature on the subject, [e.g., Heinz 1979 and Heinz et al. 2009]);

Identify, through stable isotope analysis on feathers molted at wintering sites, the wintering latitudes for the Parker River and Rachel Carson NWRs breeding populations. Parker River has already been identified as being at risk of effects;

Elucidate connectivity between other wintering and breeding sites through stable isotope analysis of samples from other collaborating researchers on the eastern seaboard and acquisition of publicly available banding-recapture data for this species from the U.S. Geological Survey Bird Banding Lab.

Methods and Materials

Sparrows were captured during the wintering period from 17 December, 2008 through 25 February, 2009 and 25 October, 2009 through 7 April, 2010 at 12 sites chosen based on likely presence (Figure 1a). Teams of 3-10 people walked abreast, dragging a 60 m weighted rope through appropriate habitat to flush birds into mist nets. Birds were removed from nets and up to 100 µl of blood was collected in two heparanized 75 micrograms per liter (µl) capillary tubes from the brachial vein punctured with a 30 1/2 gauge needle. One tube was used for mercury analysis and the other for stable isotopes. Blood was stored on ice for up to 8 hours and then frozen at -20 ºC until analysis. After morphometrics were recorded, birds were photographed and 5 breast feathers were plucked and frozen in zipped plastic bags.

Blood samples were thawed and expressed directly onto a balance before being transferred to the mercury analyzer; reported values are for wet weight. Feathers were thawed, washed with distilled water to remove particulates, dried for 48 hours in a low-humidity chamber and analyzed for mercury; these are also reported as wet weights. Blood samples used for isotopic analysis were dehydrated on a Labconco freeze-drier for 24 hours before homogenization and then packaged in crimped tin containers for shipping.

Samples were analyzed for total mercury at the College of William and Mary between 19 March, 2009 and 14 September, 2010. We used atomic absorption spectroscopy with a Milestone DMA-80 direct mercury analyzer (Shelton, CT, USA). The DMA-80 was calibrated using known standards according to machine specifications prior to the analysis and approximately every two months throughout the study period, or more often when necessary to keep standard reference material values within 7.5% of certified values. A blank, an empty sample container, a duplicate and two aliquots of each standard reference material (DORM-3 and DOLT-4) were run with every 20 samples. Two separate capillary tubes of blood from the same collection date of the same bird run on the same day were considered duplicate blood samples (n = 11 pairs of samples). Due to a paucity of sample duplicates, pairs of avian blood samples from a similar study on Tree Swallows (*Tachycineta bicolor*) were run as additional duplicates for this study (n = 13 pairs of samples). Likewise, swallow wing feathers were cut into 1 mm^2 pieces, homogenized, and used in paired aliquots for duplicate feather samples (n = 16 pairs of samples).

Relative percent difference between duplicate blood samples was 11.4 ± 10.8% (mean ± standard deviation [SD] reported throughout text, n = 24 pairs). Relative percent difference between duplicate feather samples was 7.2 ± 6.8% (n = 16 pairs). Minimum detection limit was 0.002 to 0.009 µg over the entire period of the study and all samples were above detection limit. Recovery of total Hg was 95.9% ± 3.6% for DORM-3 (n = 38) and 94.9 ± 2.1% for DOLT-4 (n = 38).

Blood for stable isotope analysis was collected simultaneous with mercury samples and analyzed at the University of California-Davis Stable Isotope Facility (Davis, CA, USA). Ratios of stable isotopes of nitrogen and carbon were measured by continuous-flow isotope ratio mass spectrometry (20-20 mass spectrometer, Sercon, Crewe, UK). The samples were combusted at 1,000°C in an on-line elemental analyzer (PDZEuropa, Sandbach, UK; ANCA-GSL, Cheshire, UK). Sample ratios were compared to those of pure cylinder gases injected into the spectrometer before and after the sample peaks. Stable isotope ratios are reported in parts per thousand (‰), in the standard delta (δ) notation, of the standards for nitrogen (atmospheric nitrogen) and carbon (Vienna PeeDee Belemnite). The equation, $\delta X = [(R_{sample}/R_{standard}) - 1] * 100$, was used to calculate values, where X is the heavier isotope, ^{15}N or ^{13}C; R_{sample} is the isotopic ratio in the sample; and $R_{standard}$ is the ratio in the standard. Measurement errors averaged ± 0.1‰.

Comparisons of mercury and isotopes for the two species were made using a two-tailed T-test. Relationships between feather and blood mercury and mercury and $\delta^{15}N$ were tested using linear regressions. A general linear model with species, site, and their interaction as factors was used to investigate differences in mercury between sampling sites. All statistics were performed in SPSS 19 (IBM).

Results and Discussion

Mercury levels for all birds combined were very similar across the two winters of the study (2008-2009: 0.24 ± 0.20 ppm, n = 77, 2009-2010: 0.26 ± 0.20 ppm, n = 178) and there was no significant difference between years so they were combined for all analyses (year: $F_{1, 254} = 0.33$, $P = 0.57$; year*species interaction $F_{1, 251} = 1.58$, $P = 0.21$). Both species and site significantly affected blood mercury concentration (Whole model $r^2 = 0.71$; Species: $F_{1, 235} = 164.20$, $P < 0.01$; Site: $F_{8, 228} = 19.91$, $P < 0.01$; site*species interaction: $F_{8, 219} = 10.22$, $P < 0.01$) such that saltmarsh sparrows had higher mercury than Nelson's sparrows at every site and absolute and relative mercury levels varied across sites (Figure 1b).

Total mercury in blood of saltmarsh sparrows on wintering sites was 0.37 ± 0.21 ppm (n = 127), almost 3x higher than that of Nelson' sparrows captured at the same sites and season (0.14 ± 0.08 ppm, n = 130). Feather mercury was approximately 2x higher in saltmarsh sparrows (6.25 ± 3.48 ppm, n = 105) than in Nelson's sparrows (2.76 ± 2.44 ppm, n = 114; $F = 20.65$, $P < 0.001$). The relationship between individual feathers and blood was weak in both species, but significant and positive in saltmarsh sparrows (saltmarsh: n = 105, $r^2 = 0.06$, $F = 6.79$, $P = 0.01$; Nelson's: n = 114, $r^2 = 0.01$, $F = 1.07$, $P = 0.30$; Figure 2).

Stable isotope analyses revealed that saltmarsh sparrows had similar $d^{15}N$ values (10.458 ± 1.348, n = 92) to Nelson's sparrows (10.672 ± 1.896 ‰, n = 90, $t = 0.88$, $P = 0.38$; Figure 3), whereas the two species differed in $d^{13}C$ (saltmarsh: -16.238 ± 2.100‰, n = 92; Nelson's: -13.859 ± 1.885, n = 90; $t = 8.04$ $P < 0.01$; Figure 3). There was a weak but significant positive

relationship between individual $d^{15}N$ and blood mercury levels in both species (saltmarsh: n = 91, $r^2 = 0.08$, $F = 7.71$, $P < 0.01$; Nelson's: n = 89, $r^2 = 0.10$, $F = 9.98$, $P < 0.01$; Figure 4).

Saltmarsh and Nelson's sparrows were recognized as separate species only in 1995, and thus biologists are only now describing the unique natural histories of each species (Greenlaw and Rising 1994). Interest in their mercury levels began in 2006 with the report that where the breeding range of the two overlapped, in Maine, USA, saltmarsh sparrows had higher blood mercury concentrations, thought to be due to their larger bill allowing them to forage at a higher trophic level (Shriver et al. 2006). Extensive sampling since that time has revealed that at many breeding sites saltmarsh sparrows bioaccumulate mercury to levels above those typically believed to harm songbirds (Lane et al. 2011). A recently published study on Nelson's Sparrows documented that at one of two previously unstudied inland populations, blood mercury levels are comparable to saltmarsh sparrow levels (North Dakota), whereas at the other site (Ontario) levels are below those originally reported for Nelson's sparrows in Maine (Winder and Emslie 2011). Thus, some populations of both species experience mercury levels of concern during the breeding season.

Both sparrow species are migratory, wintering together at numerous tidal marshes along the eastern coast of North America as well as the Gulf of Mexico. We sampled the northern portion of the shared wintering range, in the Chesapeake Bay and seaside marshes of the Delmarva Peninsula, Virginia, and found that while both species had lower blood mercury than on their breeding ranges, the relative difference was the same as originally reported for their shared breeding range, with saltmarsh sparrows having approximately three times the concentration of blood mercury. Feathers incorporate mercury from blood at the time that they are grown, thus the feathers we sampled on the winter range contained mercury from the breeding range (Condon and Cristol 2009). Not surprisingly, then, there was only a weak relationship between feather and blood mercury for either species, but the relative difference between the species was similar to that reported for blood on the shared breeding range (Shriver et al. 2006). Feather and blood mercury values of Nelson's Sparrows from North Carolina wintering sites were recently published (Winder and Emslie 2011), and are remarkably similar to the values presented in Table 2.

These results suggest that saltmarsh sparrows, which nest along the northeastern coast of North America, lower their mercury levels when they migrate to wintering grounds along the southeastern coast. Several breeding populations have mercury levels that warrant concern (Lane et al. 2011), particularly when considering the other pressures on this species (e.g. coastal "squeeze" due to real estate development and climate-induced sea level rise). However, for the majority of the year, this species experiences mercury levels that are lower than on the breeding ground. This may be due to a shift in diet downwards on the food chain, for example fewer insects and more seeds. It may also reflect lower environmental mercury levels. Bald Eagles (*Haliaeetus leucocephalus*) have lower feather mercury levels in the Chesapeake Bay than do those in any other North American population thus far reported (Cristol et al. 2012). Regardless of the mechanism, saltmarsh sparrow blood mercury is two to three times higher on the breeding

grounds, and this is where any effort at ameliorating the mercury exposure threat should be focused.

In contrast to saltmarsh sparrows, Nelson's sparrows nesting in eastern North America (*A. n. alterus and A. n. subvirgatus*) appear to face little threat from mercury exposure, based on currently available data. However, the central population (*A. n. nelsoni*), which was recently sampled in North Dakota, deserves a closer look (Table 2). The elevated levels reported from 24 individuals at a single site are cause for alarm, but conclusions about this population require more expansive sampling throughout the large breeding range in the Great Plains of Canada and USA.

While mercury levels of Nelson's sparrows on the wintering grounds may have no immediate implications for conservation of this species, due to their generally low concentrations year-round, they are interesting when compared to the higher levels in the closely-related saltmarsh sparrow. One hypothesis for the consistent difference between the two species is that saltmarsh sparrows, with their larger bill, eat larger prey that occupy a higher trophic position, and thus experience more biomagnification of mercury (Shriver et al 2006). To test this hypothesis, we compared signatures of stable isotopes of nitrogen, a commonly used proxy for trophic position (Kelly 2000). The two species had very similar nitrogen signatures in blood sampled on the wintering ground, suggesting that during the non-breeding season they eat diets that are equivalent in terms of trophic position.

In contrast, saltmarsh sparrows were relatively enriched in the heavier carbon isotope, suggesting reliance on a food web with more plants that use C4 photosynthesis, such as *Spartina* grasses, as opposed to plants using C3 photosynthesis, such as *Juncus* rushes or woody shrubs. While little is known about habitat segregation in these two cryptic sparrow species, the difference in carbon stable isotopic signatures suggests that they sit atop different food chains in winter, at the base of which are different plants. This could occur in the same microhabitat through direct consumption of different seed species. The difference in carbon stable isotope signatures could also arise through habitat segregation leading to consumption of different seeds or invertebrates that have previously eaten different types of plants. Spatial segregation could be the direct explanation for the higher mercury level of saltmarsh sparrows, if, for example, more methylation occurs in sections of saltmarsh covered with *Spartina* than *Juncus* and shrubs. Alternately, the species' food chains may have differential terrestrial versus marine inputs. Further study will be necessary to link mercury to sparrow diet components and to track those to their source in the habitat.

Saltmarsh sparrows have higher mercury levels than Nelson's sparrows on the wintering ground, as previously reported for the breeding grounds. The difference in mercury levels is not explained by feeding at different trophic levels, as previously suggested; rather, they may feed from food webs with different carbon sources, as would occur if they segregated between high (shrubby) and low (grassy) saltmarsh in winter. Saltmarsh sparrows, which face a potential threat

from elevated mercury levels on the breeding grounds, have lower mercury after they migrate to wintering grounds, which should reduce the fitness consequences of breeding season mercury.

Management Action(s)

This study showed that sparrows breeding at mercury-impacted NWRs, such as Parker River NWR, may be able to reduce mercury levels during the winter because few are exposed during that season. However, mercury was still present in both species on the wintering grounds, and all populations of these two newly-recognized species of conservation concern should be monitored periodically as global mercury levels change. The study showed that the overall threat level of mercury in general was lowered because mercury at wintering sites was lower, thus allowing more targeted management actions at breeding sites where mercury may be having localized effects.

Future studies should be aimed at:

Assessment of whether mercury is having effects (reproductive impairment, shortened lifespan) on breeding populations;

Expand mercury monitoring of saltmarsh and Nelson's sparrows at wintering grounds further south.

Planning remedial actions, such as vegetation management to reduce methylation or redirection of contaminated waterways to reduce inputs, or, alternatively, application of triage if the population is not sustainable on a particular NWR in the face of climate-induced sea level rise or other impending threats.

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Tables and Figures

Table 1. Rangewide saltmarsh sparrow mercury concentrations (parts per million [ppm]).

Location	Blood Hg (n) (range[a])	Feather Hg (n) (range[a])	Season	Source
Maine	0.69 (n = 53) (0.56-0.87)	----------	Breeding	Shriver et al. 2006
Maine	0.64 (n = 229) (0.31-0.85)	----------	Breeding	Lane et al. 2011
New Hampshire	0.74 (n = 95) (0.32-1.10)	----------	Breeding	Lane et al. 2011
Massachusetts	1.37 (n = 160) (0.88-1.80)	----------	Breeding	Lane et al. 2011
Rhode Island	0.80 (n = 81) (0.59-1.10)	----------	Breeding	Lane et al. 2011
Connecticut	0.50 (n = 31) (0.24-0.61)	----------	Breeding	Lane et al. 2011
New York	1.01 (n = 44) (0.68-1.50)	----------	Breeding	Lane et al. 2011
Delaware	0.48 (n=35) (0.40-0.54)	----------	breeding	Warner 2009
Virginia	0.37 (n=127) (0.15-0.68)	6.25 (n = 105) (2.96-10.76)	winter	This study

[a]Range represents minimum and maximum site averages, except if sampled at one site, as for North Dakota and Ontario, where range represents minimum and maximum individuals.

Table 2. Rangewide Nelson's sparrow mercury concentrations.

Location	Blood Hg (n) (range[a])	Feather Hg (n) (range[a])	Season	Source
Ontario	0.22 (n = 13) (0.14-0.36)	1.21 (n = 14) (0.47-5.72)	breeding	Winder and Emslie 2011
North Dakota	1.07 (n = 24) (0.68-1.87)	0.98 (n = 24) (0.34-3.19)	breeding	Winder and Emslie 2011
Maine	0.41 (n = 28) (0.26-0.56)	----------	breeding	Shriver et al. 2006
Virginia	0.14 (n = 130) (0.09-0.20)	2.76 (n = 114) (1.81-4.74)	winter	This study
North Carolina	0.14 (n = 47) (0.11-0.16)	2.94 (n = 55) (2.57-3.80)	winter	Winder and Emslie 2011

[a]Range represents minimum and maximum site averages, except if sampled at one site, as for North Dakota, and Ontario, where range represents minimum and maximum individuals.

Figure 1a) Sampling locations and b) associated average blood total mercury levels for Nelson's sparrows (black bars) and saltmarsh sparrows (white bars). Error bars are one standard error. Numbers refer to sites and are described in Appendix 1.

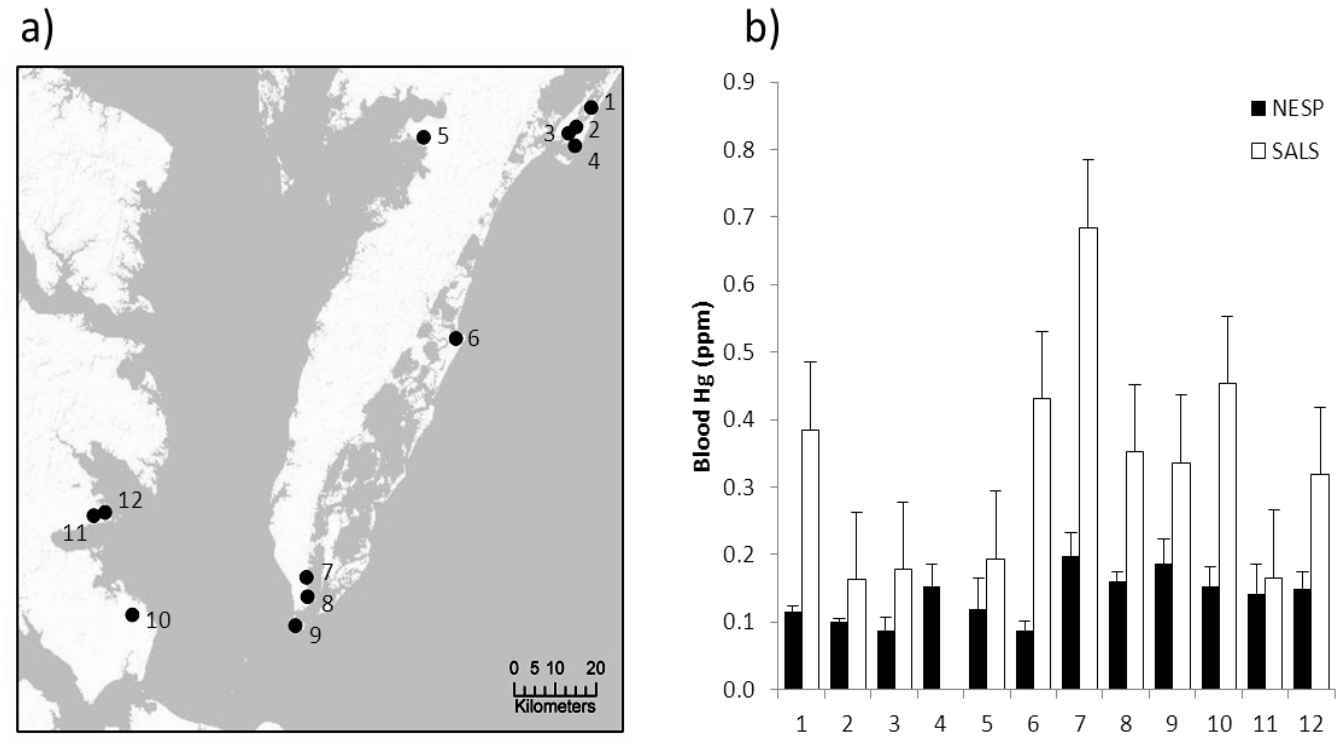


Figure 2. Correlations between blood and feather mercury levels for Nelson's sparrow (o) and saltmarsh sparrow (Δ) sampled in Virginia wintering range. Lines are linear regressions for each species.

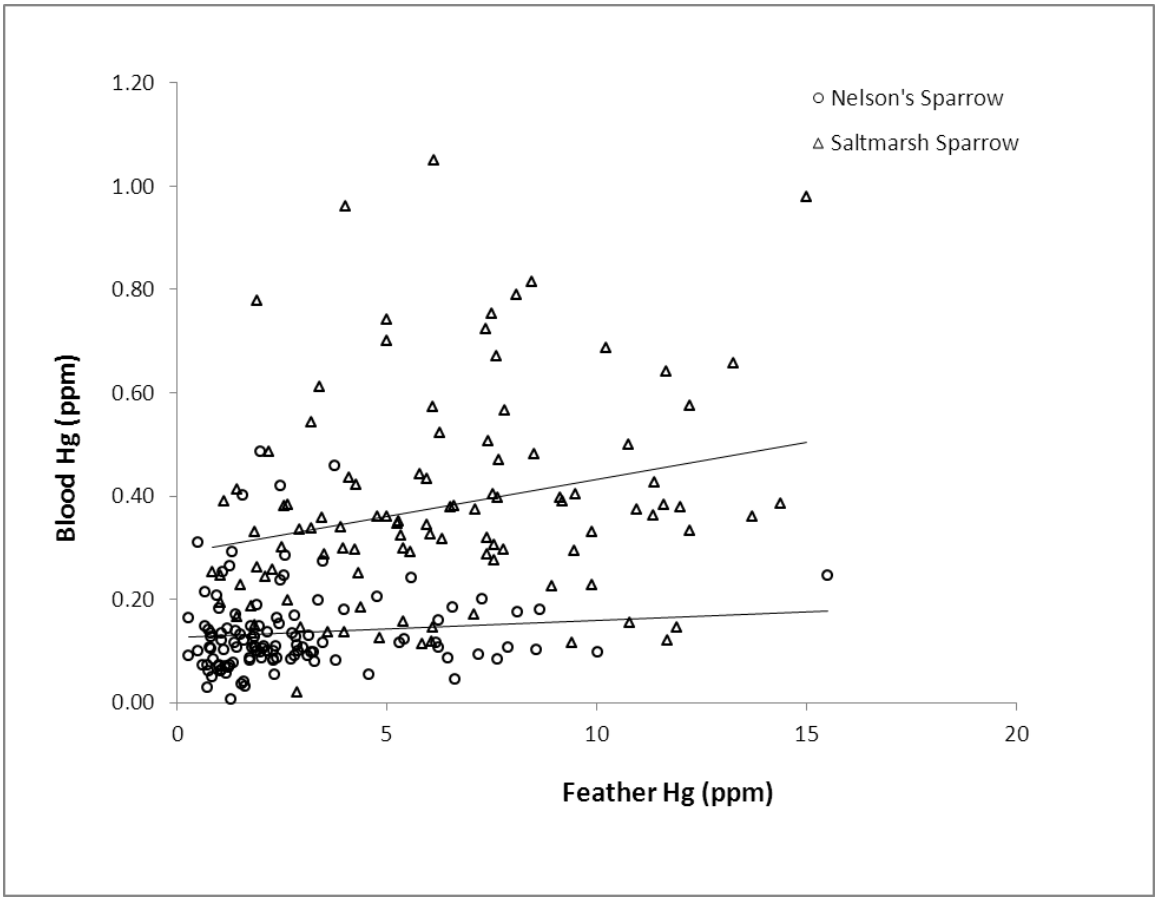


Figure 3. Stable isotope values for Nelson's sparrow (o) and saltmarsh sparrow (Δ). Small symbols are individual measures and large symbols are species average values with error bars representing one standard deviation.

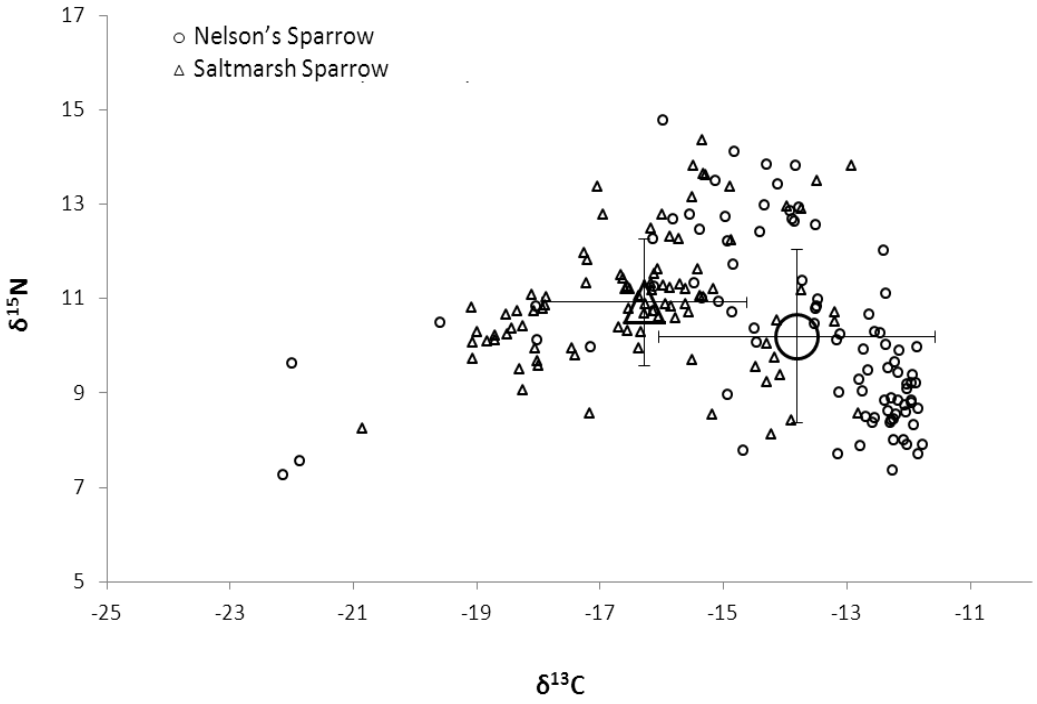
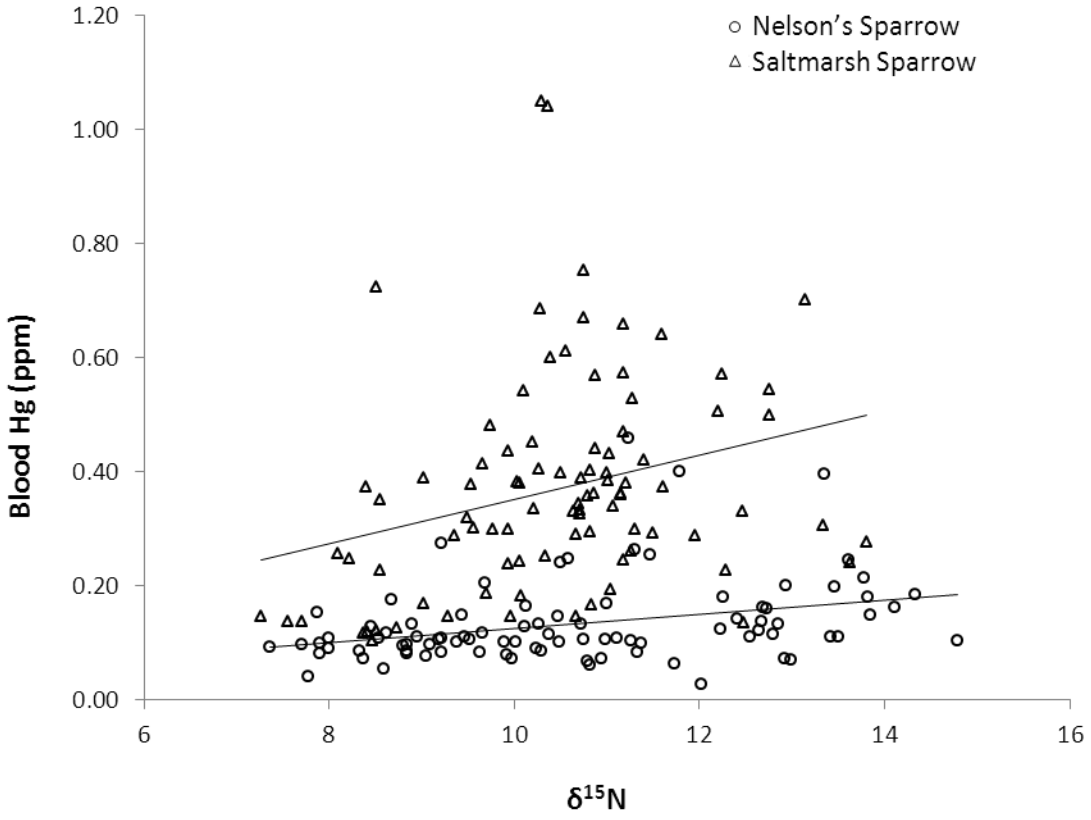


Figure 4. Correlations between nitrogen stable isotope enrichment and mercury level for Nelson's sparrow (o) and saltmarsh sparrow (Δ). Lines are linear regressions for each species.



Appendix 1

Site #	Location name	Latitude	Longitude
1	Assateague Bay	37.954654	-75.317580
2	Chincoteague	37.922407	-75.350723
3	Smalley Drain	37.912024	-75.367448
4	Tom’s Cove	37.891455	-75.353260
5	Belinda	37.905207	-75.685916
6	Parramore Island	37.572239	-75.614844
7	Magotha	37.174903	-75.943232
8	Bull’s Drive	37.142255	-75.941172
9	Fishermans Island	37.094062	-75.967886
10	Poquoson	37.111965	-76.325995
11	Maryus	37.277605	-76.410803
12	Monday Creek	37.282993	-76.385990